*Phytologia* (November 1995) 79(5):325-339.

# *MUHLENBERGIA MONTANA* AND *M. QUADRIDENTATA*, A CASE OF A NATURAL HYBRID SWARM

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## ABSTRACT

This study examined variation in morphology for 49 populations of *Muhlenbergia montana* (Nutt.) Hitchc. and *M. quadridentata* (H.B.K.) Kunth representing the sympatric range of the species. These and previous results suggest the formation of hybrid swarms between the two species. Suspected hybridization is confirmed by the morphometric analysis of the species growing in this area. Flavonoid profiles, anatomical, and cytological features seem to support this theory.

KEY WORDS: *Muhlenbergia montana*, *Muhlenbergia quadridentata*, hybrid swarm, Poaceae

## RESUMEN

El presente estudio examinó la variación morfológica de 49 poblaciones de *Muhlenbergia montana* (Nutt.) Hitchc. y *M. quadridentata* (H.B.K.) Kunth, representando el área de distribución simpátrica de las especies. Estos y previos resultados sugieren la formación de camadas de híbridos entre las dos especies. La hibridación sospechada se confirma a través del análisis morfométrico de las especies que ocurren en esta área. El perfil de flavonoides y los caracteres anatómicos y citológicos parecen apoyar esta teoría.

PALABRAS CLAVES: *Muhlenbergia montana*, *Muhlenbergia quadridentata*, camadas de híbridos, Poaceae

*Muhlenbergia montana* (Nutt.) Hitchc., a widely distributed species (from Montana to México and Guatemala) is perhaps, a very successful species that along its wide distribution seems to hybridize with putative species; Welsh *et al.* (1987)

reported numerous intermediates formed with *M. filiculmis* Vasey in the Rocky Mountains, while, Herrera-Arrieta & Grant (1993) mention the suspected hybridization with *M. quadridentata* (H.B.K.) Kunth, in the western and central part of México.

Initial macromorphological studies of the *Muhlenbergia montana* complex (Herrera-A. & Bain 1991 and Herrera-Arrieta & Grant 1993, 1994) have shown that some specimens of *M. quadridentata* shared certain features of *M. montana*. Further macromorphological observations of floral and vegetative characters analyzed with multivariate statistical tests and phenetic analyses, augmented by information on pollen fertility and their geographical range of distribution, provide a context for evaluating the taxonomic limits and relationships of these two species.

Phenetic analyses of morphological, anatomical, and flavonoid content data suggested that *Muhlenbergia montana* and *M. quadridentata* hybridize in sympatric areas.

The present analysis attempted to estimate the morphological variation among populations within these two species belonging to the sympatric area of distribution in México, to confirm that hybridization occurs.

## MATERIALS AND METHODS

A study of 49 freshly collected specimens (Table 1) was assembled. The collection locations seemed to cover the sympatric area of distribution of these two species at a range of 2100-3650 m, 17-24° N and 90-105° W. A few other herbarium specimens were selected to represent the morphological variation.

Populations of *Muhlenbergia* vary in size from a few scattered individuals to hundreds of plants covering a large area. Sample sizes were randomly selected of ten to fifteen individuals per population at each location, to maximize the probability of sampling genetically different individuals. Eighteen morphological characters were scored and are shown in Table 2. Many loaned herbarium specimens were reviewed from: CIIDIR, CHAPA, ENCB, HUAA, IEB, and MEXU (acronyms follow Holmgren *et al.* 1990).

The individuals of each population were measured for eighteen continuous macromorphological variables (Table 2), where each OTU is represented by the mean value (measurement) per variable. Application of Cluster Analysis and Principal Component Analysis (PCA) for a phenetic study were the most representative and are elaborated below.

A distribution map (Figure 1) is included, based on over 950 herbarium specimens identified as *Muhlenbergia montana* and *M. quadridentata*. After examination, 49 locations were selected to collect fresh material representing the geographic ranges and putative hybrids. The specimens were measured and recorded.

Table 1. Specimens of *Muhlenbergia* analyzed in this study.

*M. montana* (Nutt.) Hitchc. MEX. AGS: *De La Cerda 3989* (CIIDIR,HUAA). CHIH: *Herrera, Peterson, & Annable 950, 956, 964, 968, 970, 972, 974, 980* (CIIDIR,MTMG,US); *Peña 802* (CIIDIR). DF: *Herrera & Cortés 922, 924* (CIIDIR, MTMG,US). DGO: *Herrera & Acevedo 984* (CIIDIR,MTMG,US); *Acevedo 582* (CIIDIR); *Acevedo & González 529, 534, 535, 536, 537, 540* (CIIDIR); *Herrera & González 1022* (CIIDIR). MOR: *Herrera & Cortés 926, 928* (CIIDIR,MTMG,US). OAX: *Herrera 900* (CIIDIR,MTMG); *Carrillo 361* (MEXU,ENCB).

*M. quadridentata* (H.B.K.) Kunth MEX. COL: *Herrera & Cortés 935, 936* (CIIDIR,MTMG,US). DF: *Herrera & Cortés 911* (CIIDIR,MTMG,US). DGO: *Acevedo & González 527* (CIIDIR,MTMG). HGO: *Chavez 134* (CIIDIR,ENCB); *Mancera 1* (CIIDIR,CHAPA). JAL: *Herrera & Cortés 933* (CIIDIR,MTMG,US). MEX: *Herrera & Cortés 904, 906, 907, 908, 913, 914, 915, 929* (CIIDIR,MTMG,US); *Herrera 241* (CIIDIR,ENCB); *Vega 276* (CIIDIR, CHAPA,ENCB); *Hernández 15/78* (CIIDIR,ENCB). MOR: *Herrera & Cortés 925, 927* (CIIDIR,MTMG,US). PUE: *Herrera & Cortés 916, 917, 918, 919* (CIIDIR,MTMG,US). OAX: *Herrera 899* (CIIDIR,MTMG).

Table 2. Coding of macromorphological variables used in the phenetic analysis.

1. Leaves length, num (for numerical).
2. Old sheaths, 1) present, 2) absent.
3. Lamina leaves, 1) involute, 2) flat, 3) flat-involute.
4. Leaf width, num.
5. Ligule length, num.
6. Ligule shape, 1) truncate, 2) apiculate.
7. Spikelets length, num.
8. First glume length, num.
9. Second glume length, num.
10. First glume width, num.
11. Second glume width, num.
12. Second glume teeth length, num.
13. Lemma length, num.
14. Lemma pubscence, 1) in base and margins, 2) in the whole surface.
15. Lemma awn length, num.
16. Palea length, num.
17. Palea pubescence, 1) scarce, 2) moderate.
18. Anthers length, num.

Flavonoid profiles were taken from (Herrera-A. & Bain 1991). A data matrix of morphological characters (Tables 3 & 4) was submitted to a Principal Component Analysis (Figures 2-4) and a cluster analysis using the unweighted pair-group mathematical average clustering analysis (UPGMA) of the Canberra distance matrix through the use of the Multivariate Statistical Package Version 1.31, Kovach (1987) to generate the dendrogram (Figure 5).

Differential staining (Alexander 1969) of aborted and nonaborted spores was used to assess sterility in the suspected hybrid populations, results are presented in Table 5.

## RESULTS AND DISCUSSION

*Muhlenbergia quadridentata* is often confused with *M. montana* (McVaugh 1983; Herrera-A. & Bain 1991; Herrera-Arrieta & Grant 1992). Field observations of the Mexican populations of these two species suggest that, although the two taxa can often be easily recognized in the field, variation between the distinguishing characters and the presence of intermediate forms have caused confusion in this group.

*Muhlenbergia montana* is more widely distributed, at elevations from 2000 to 3100 m, from 15° to 45° N and 90° to 112° W. *Muhlenbergia quadridentata* grows mostly at higher altitudes (more than 3000 m), and from 17° to 21° N, 96° to 102° W. Scattered populations were found close to 2000 m, at higher latitudes 24° N and 105° W. The former has spikelets and anthers shorter than *M. quadridentata*, with glumes subequal, and the second glume 3-toothed and conspicuously but shortly 3-awned.

These two species seem to form a group on the basis of their flavonoid content (Herrera-A. & Bain 1991). The flavonoid profiles show that *Muhlenbergia montana* lacks four compounds present in *M. quadridentata*; while *M. quadridentata* lacks a compound always present in *M. montana*. These unique compounds are considered diagnostic marks (mark-q and mark-m for the compounds present in one species and absent in the other) in this work. From the twenty populations of *M. montana* studied for flavonoids (Herrera-A. & Bain 1991), fourteen shared having the well defined compounds identified for this species. On the other side, from the seventeen populations studied of *M. quadridentata*, fourteen shared having the seventeen flavonoids characteristic for *M. quadridentata*. The remaining populations (six populations of the former and three populations of the later) have shown a variable mixture of the marked flavonoids. The presence or absence of these compounds revealed key characters to delineate the identity of morphological intermediates between *M. montana* and *M. quadridentata*.

Principal Component Analysis (PCA), using averages of eighteen measured characters (Table 2), was used to produce a graphic representation of the variation among the groups (Figures 2 to 4). Relative positions of individuals on the PC axes represent their relative similarity for the characters used. In this analysis the two species are completely separated by the first two principal components.

Table 3. Data matrix for the characters of *Muhlenbergia quadridentata* (H.B.K.) Kunth used in this study.

| OTU | Coll. # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H&C 899 | 30 | 2 | 3 | 2.0 | 2.0 | 1 | 4.0 | 2.8 | 4.0 | 0.7 | 1.0 | 1.5 | 3.5 | 1 | 1.0 | 3.0 | 1 | 1.8 |
| 2 | H&C 900 | 20 | 2 | 1 | 1.5 | 2.0 | 1 | 4.0 | 2.0 | 3.0 | 0.7 | 1.2 | 0.6 | 3.5 | 1 | 1.0 | 3.0 | 1 | 1.8 |
| 3 | H&C 933 | 18 | 2 | 1 | 1.0 | 2.0 | 1 | 4.0 | 1.8 | 3.5 | 0.7 | 1.0 | 0.0 | 3.5 | 1 | 1.0 | 3.0 | 1 | 1.6 |
| 4 | VEGA 276 | 22 | 2 | 1 | 1.0 | 3.0 | 2 | 4.0 | 2.0 | 3.6 | 0.6 | 1.0 | 0.4 | 3.4 | 1 | 0.9 | 3.0 | 1 | 2.0 |
| 5 | H&C 914 | 28 | 2 | 1 | 1.0 | 2.0 | 1 | 4.2 | 1.8 | 3.2 | 0.5 | 1.0 | 0.8 | 4.2 | 1 | 1.1 | 3.5 | 1 | 2.2 |
| 6 | H&C 906 | 33 | 2 | 3 | 2.0 | 2.0 | 2 | 4.5 | 2.0 | 4.0 | 0.6 | 1.0 | 1.0 | 4.0 | 1 | 1.4 | 3.5 | 1 | 2.1 |
| 7 | H&C 917 | 23 | 2 | 1 | 0.8 | 2.0 | 1 | 3.8 | 1.6 | 3.2 | 0.7 | 0.8 | 0.0 | 3.6 | 1 | 0.8 | 3.0 | 1 | 1.6 |
| 8 | H&C 916 | 30 | 2 | 1 | 1.0 | 2.0 | 1 | 4.0 | 1.7 | 3.0 | 0.5 | 1.0 | 0.2 | 3.8 | 1 | 0.8 | 3.0 | 1 | 2.2 |
| 9 | H&C 918 | 30 | 2 | 1 | 1.0 | 2.0 | 1 | 4.8 | 2.8 | 4.8 | 0.4 | 1.2 | 0.1 | 4.2 | 1 | 1.2 | 4.0 | 1 | 2.2 |
| 10 | H&C 913 | 22 | 2 | 1 | 1.0 | 1.5 | 1 | 3.5 | 2.0 | 3.0 | 0.7 | 1.4 | 0.1 | 3.2 | 1 | 0.8 | 3.0 | 2 | 2.1 |
| 11 | H&C 919 | 30 | 2 | 1 | 1.0 | 2.0 | 1 | 3.8 | 1.4 | 2.8 | 0.7 | 1.4 | 0.5 | 3.8 | 1 | 0.7 | 3.5 | 2 | 1.8 |
| 12 | H&C 915 | 21 | 2 | 3 | 2.0 | 2.0 | 1 | 5.0 | 2.0 | 4.0 | 0.8 | 1.4 | 0.5 | 4.5 | 1 | 0.8 | 3.5 | 1 | 2.2 |
| 13 | H&C 927 | 18 | 2 | 3 | 2.0 | 4.0 | 2 | 3.8 | 1.2 | 2.8 | 0.5 | 1.0 | 0.3 | 3.2 | 1 | 1.2 | 3.0 | 2 | 2.2 |
| 14 | H&C 911 | 28 | 2 | 3 | 2.0 | 4.0 | 2 | 3.5 | 1.5 | 2.5 | 0.4 | 0.8 | 0.2 | 3.0 | 1 | 1.0 | 2.5 | 1 | 1.8 |
| 15 | H&C 907 | 13 | 2 | 1 | 1.5 | 2.0 | 1 | 3.8 | 2.0 | 3.0 | 0.5 | 1.2 | 0.1 | 3.5 | 1 | 1.0 | 3.0 | 1 | 2.0 |
| 16 | H&C 904 | 15 | 2 | 1 | 1.0 | 2.0 | 1 | 3.8 | 2.0 | 3.0 | 0.6 | 1.0 | 0.1 | 3.5 | 1 | 0.9 | 3.0 | 2 | 1.8 |
| 17 | H&C 936 | 16 | 2 | 1 | 1.0 | 4.0 | 2 | 4.0 | 1.5 | 2.8 | 0.8 | 1.2 | 0.1 | | 1 | 0.8 | 3.8 | 1 | 2.0 |
| 18 | H&C 935 | 12 | 2 | 1 | 1.0 | 3.0 | 2 | 4.2 | 2.2 | 3.8 | 0.7 | 1.0 | 0.0 | 4.0 | 1 | 1.2 | 3.8 | 2 | 2.0 |
| 19 | H&C 929 | 21 | 2 | 1 | 1.0 | 2.0 | 1 | 3.2 | 1.6 | 2.8 | 0.7 | 1.1 | 0.1 | 3.0 | 1 | 0.8 | 2.8 | 1 | 1.6 |
| 20 | H&C 908 | 20 | 2 | 1 | 1.0 | 2.0 | 1 | 4.0 | 2.2 | 3.5 | 0.5 | 1.0 | 0.5 | 3.5 | 1 | 0.9 | 3.2 | 1 | 1.5 |
| 21 | H&C 925 | 15 | 2 | 1 | 1.0 | 10 | 2 | 5.0 | 2.5 | 4.0 | 0.4 | 0.8 | 0.2 | 4.5 | 1 | 1.2 | 4.0 | 2 | 2.2 |
| 22 | CHAVEZ 134 | 11 | 2 | 1 | 1.0 | 8.0 | 2 | 4.0 | 1.8 | 3.0 | | 1.4 | 0.1 | 3.8 | 1 | 0.8 | 3.2 | 1 | 2.0 |
| 23 | HDEZ 15/78 | 20 | 2 | 1 | 2.0 | 2.5 | 1 | 4.2 | 2.2 | 3.8 | 0.7 | 1.2 | 0.3 | 4.0 | 1 | 0.4 | 3.8 | 1 | 1.8 |
| 24 | MANCERA 1 | 19 | 2 | 2 | 2.0 | 2.0 | 1 | 3.2 | 2.0 | 3.0 | 0.7 | 1.2 | 0.3 | 3.0 | 1 | 1.2 | 2.8 | 2 | 1.8 |
| 25 | H 241 | 11 | 2 | 1 | 1.0 | 2.0 | 1 | 3.2 | 1.8 | 2.8 | 0.6 | 0.8 | 0.5 | 3.0 | 1 | 1.2 | 2.8 | 2 | 2.0 |

COLLECTORS= H&C: Herrera & Cortés, HDEZ: Hernández, H: Herrera.

Table 4. Data matrix for the characters used in this study of *Muhlenbergia montana* (Nutt.) Hitchc.

| OTU | Coll. # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | ACEV 582 | 22 | 2 | 2 | 1.7 | 2.2 | 1 | 4.5 | 4.0 | 5.0 | 0.7 | 1.0 | 2.5 | 4.2 | 2 | 1.2 | 4.0 | 2 | 2.0 |
| 27 | H&G 1022 | 27 | 2 | 1 | 1.2 | 2.5 | 2 | 3.8 | 2.5 | 3.0 | 0.6 | 0.9 | 1.5 | 3.5 | 2 | 1.7 | 3.0 | 2 | 2.0 |
| 28 | DLC 3839 | 27 | 2 | 3 | 1.5 | 7.0 | 2 | 3.8 | 3.0 | 3.0 | 0.6 | 1.1 | 1.5 | 3.0 | 2 | 1.2 | 2.8 | 2 | 2.0 |
| 29 | A&G 534 | 32 | 1 | 2 | 4.0 | 5.0 | 2 | 4.0 | 3.2 | 4.0 | 0.7 | 1.2 | 2.0 | 4.0 | 2 | 1.5 | 3.8 | 2 | 2.0 |
| 30 | A&G 529 | 27 | 1 | 3 | 1.5 | 4.0 | 2 | 4.0 | 3.0 | 3.8 | 0.7 | 1.2 | 1.5 | 3.8 | 2 | 1.2 | 3.5 | 2 | 1.8 |
| 31 | A&G 535 | 26 | 1 | 2 | 2.2 | 2.5 | 2 | 4.0 | 3.0 | 3.0 | 0.6 | 1.0 | 1.2 | 3.8 | 2 | 1.5 | 3.5 | 2 | 2.2 |
| 32 | CARR 361 | 20 | 2 | 1 | 1.0 | 6.0 | 2 | 3.8 | 3.0 | 3.2 | 0.5 | 0.8 | 1.8 | 3.2 | 2 | 1.5 | 3.0 | 2 | 1.6 |
| 33 | A&G 540 | 21 | 1 | 2 | 1.5 | 3.0 | 2 | 3.5 | 2.0 | 3.0 | 0.6 | 1.2 | 1.0 | 3.2 | 2 | 1.2 | 3.0 | 2 | 2.2 |
| 34 | PENA 802 | 22 | 1 | 2 | 2.5 | 1.5 | 1 | 4.0 | 3.0 | 4.0 | 0.7 | 1.2 | 2.0 | 4.0 | 2 | 1.0 | 3.8 | 2 | 0.0 |
| 35 | H&C 924 | 25 | 2 | 3 | 1.2 | 3.0 | 2 | 4.0 | 2.0 | 3.0 | 0.5 | 0.9 | 0.5 | 3.8 | 1 | 1.2 | 3.5 | 1 | 0.0 |
| 36 | H,P&A 950 | 21 | 2 | 3 | 1.5 | 10 | 2 | 4.0 | 3.5 | 3.5 | 0.4 | 0.9 | 1.5 | 4.0 | 2 | 1.0 | 3.8 | 2 | 2.0 |
| 37 | H&C 928 | 14 | 2 | 1 | 0.5 | 2.0 | 1 | 3.5 | 1.8 | 2.0 | 0.5 | 0.9 | 0.3 | 3.2 | 1 | 1.2 | 3.0 | 1 | 0.0 |
| 38 | H,P&A 968 | 16 | 1 | 1 | 1.0 | 5.0 | 2 | 3.2 | 2.0 | 2.8 | 0.5 | 0.8 | 1.5 | 3.0 | 2 | 1.5 | 2.8 | 2 | 1.8 |
| 39 | H&C 922 | 10 | 2 | 1 | 0.5 | 6.0 | 1 | 3.8 | 2.0 | 3.0 | 0.7 | 1.2 | 1.0 | 3.5 | 1 | 1.2 | 3.2 | 1 | 2.0 |
| 40 | H,P&A 972 | 22 | 2 | 1 | 1.2 | 8.0 | 2 | 4.0 | 2.8 | 3.3 | 0.6 | 1.2 | 1.6 | 4.0 | 2 | 1.1 | 3.8 | 2 | 1.8 |
| 41 | H&C 926 | 18 | 2 | 1 | 1.0 | 2.0 | 1 | 3.0 | 1.5 | 2.2 | 0.6 | 1.0 | 0.3 | 3.0 | 1 | 0.8 | 2.8 | 1 | 2.0 |
| 42 | H,P&A 970 | 21 | 2 | 3 | 1.2 | 15 | 2 | 4.0 | 3.0 | 3.5 | 0.6 | 1.0 | 1.5 | 3.5 | 2 | 0.8 | 3.2 | 1 | 2.0 |
| 43 | H,P&A 974 | 24 | 1 | 3 | 1.5 | 10 | 2 | 4.0 | 3.0 | 3.5 | 0.7 | 1.0 | 1.5 | 3.8 | 2 | 1.5 | 3.5 | ? | 1.8 |
| 44 | H,P&A 980 | 09 | 1 | 3 | 2.0 | 7.0 | 2 | 4.2 | 3.5 | 3.8 | 0.6 | 1.2 | 2.0 | 4.0 | 2 | 1.1 | 3.8 | 2 | 2.0 |
| 45 | H,P&A 956 | 21 | 2 | 3 | 2.0 | 11 | 2 | 4.0 | 3.0 | 3.5 | 0.6 | 0.9 | 1.5 | 3.8 | 2 | 1.5 | 3.5 | 2 | 1.8 |
| 46 | H,P&A 964 | 11 | 2 | 1 | 1.0 | 10 | 2 | 3.5 | 2.2 | 2.5 | 0.6 | 1.1 | 1.2 | 3.2 | 2 | 0.8 | 3.0 | 2 | 1.8 |
| 47 | H&A 984 | 11 | 1 | 1 | 0.8 | 3.0 | 1 | 3.8 | 2.0 | 2.5 | 0.6 | 1.0 | 0.5 | 3.5 | 2 | 0.8 | 3.3 | 2 | 1.5 |
| 48 | A&G 536 | 28 | 1 | 3 | 2.0 | 3.0 | 2 | 3.8 | 2.5 | 3.2 | 0.8 | 1.1 | 1.5 | 3.5 | 2 | 1.2 | 3.2 | 2 | 2.2 |
| 49 | A&G 537 | 22 | 1 | 2 | 2.5 | 2.5 | 2 | 4.0 | 2.5 | 3.5 | 0.8 | 1.2 | 0.8 | 3.8 | 2 | 1.5 | 3.5 | 2 | 1.5 |

COLLECTORS= ACEV.: Acevedo; H&C: Herrera & Cortés; H&G: Herrera & González; A&G: Acevedo & González; CARR.: Carrillo; DLC: De La Cerda; H,P&A: Herrera, Peterson & Annable.


Figure 1. Map of *Muhlenbergia montana* and *M. quadridentata* distribution.

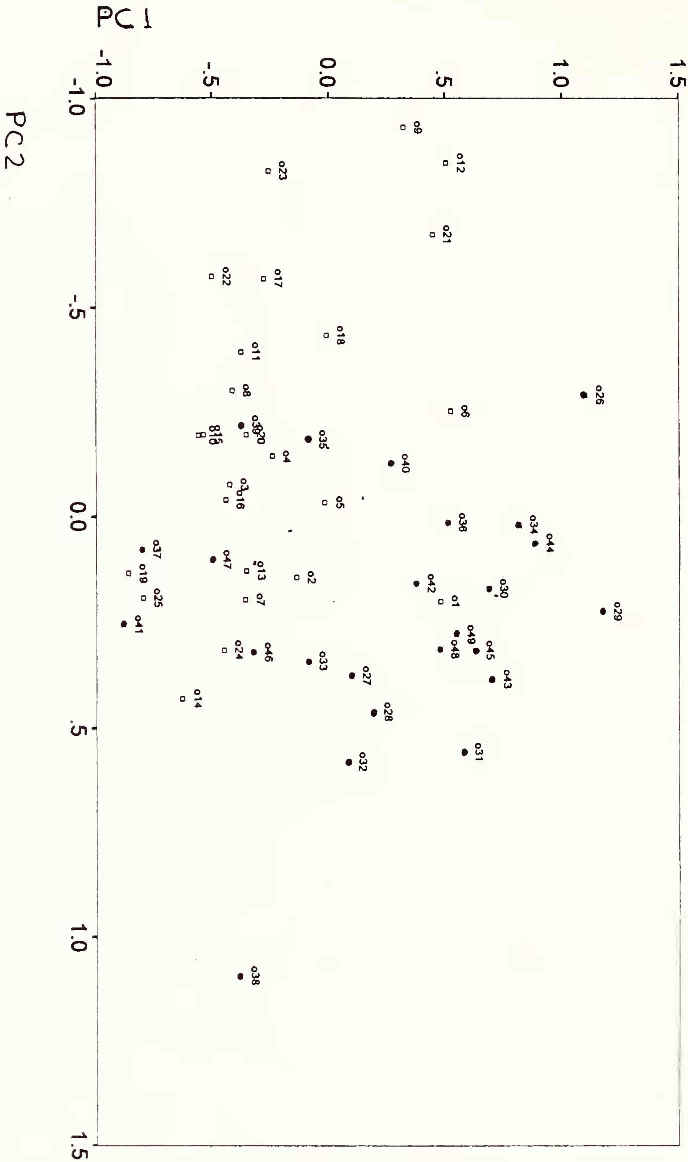


Figure 2. Scatter diagram of individuals from pure and mixed populations of *Muhlenbergia montana* and *M. quadridentata* on Principal Components 1 and 2. Grouping is based on morphological characters.

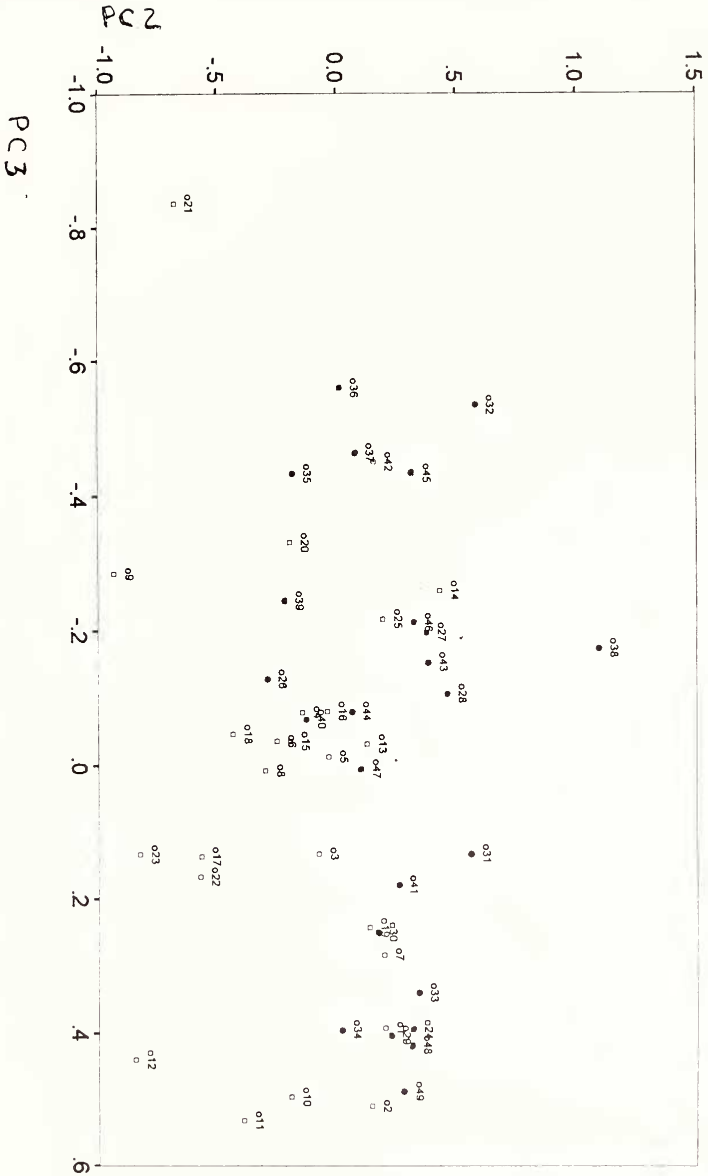


Figure 3. Scatter diagram of individuals from pure and mixed populations of *Muhlenbergia montana* and *M. quadridentata* on Principal Components 2 and 3. Grouping is based on morphological characters.

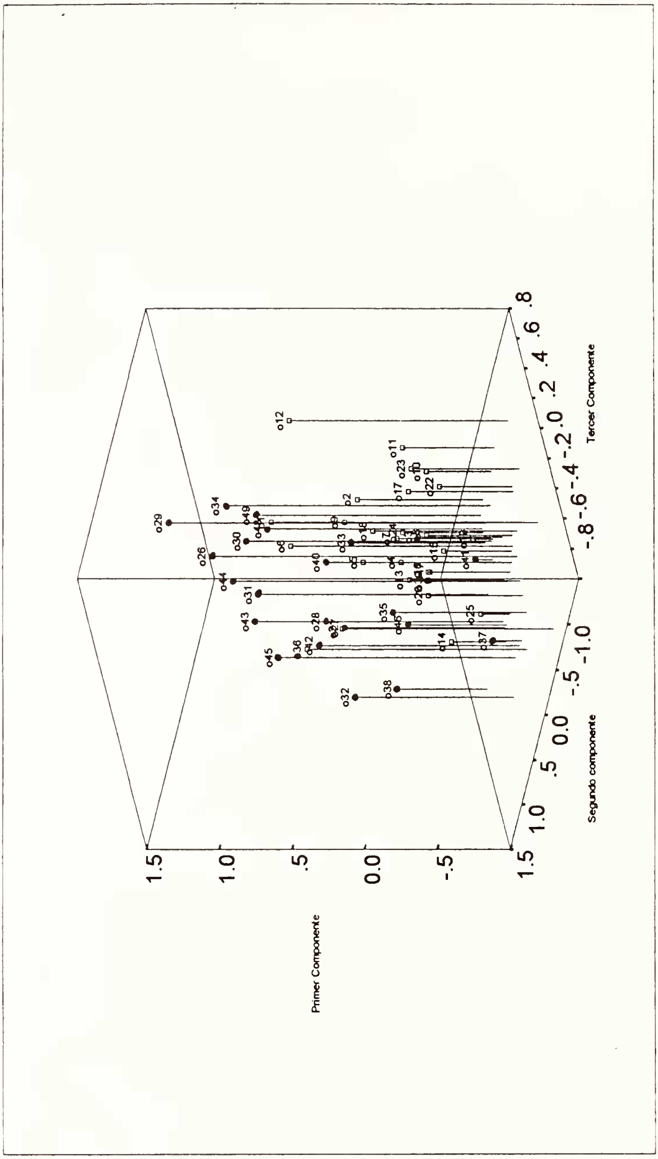


Figure 4. Scatter diagram of individuals from pure and mixed populations of *Muhlenbergia montana* and *M. quadridentata* on Principal Components 1, 2, and 3. Grouping is based on morphological characters.

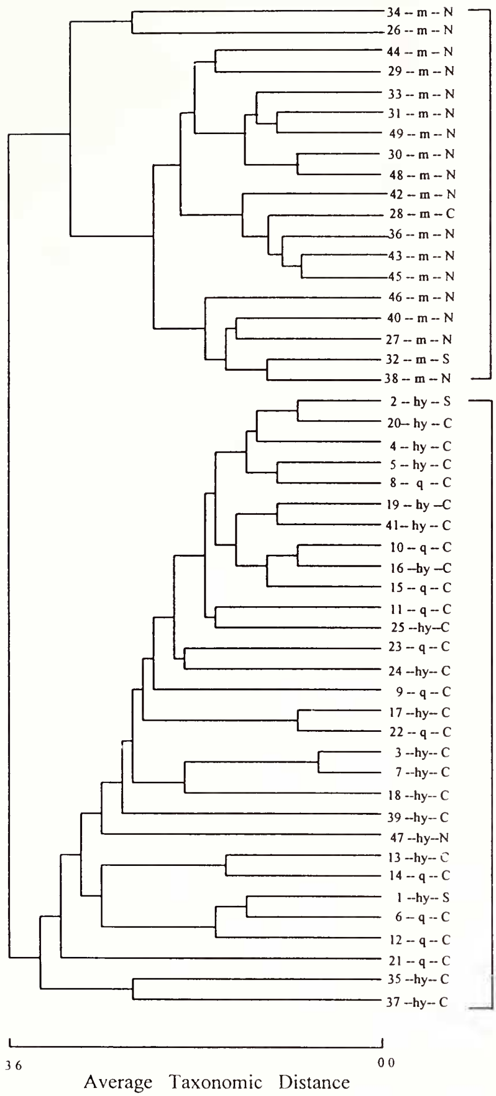


Figure 5. Phenetic relationships among accessions of *Muhlenbergia montana* and *M. quadridentata* as reflected by the cluster analysis (UPGMA) of the Canberra distance (Kovach 1987). Cophenetic correlation 0.923. Population numbers correspond to those in Tables 3 & 4. N= Northern populations, C= Central populations, and S= Southern populations.

Table 5. Percentage of pollen sterility in suspected hybrids.

| OTU | Collector # | Locality | Altitude | Good Pollen | Aborted Pollen |
|---|---|---|---|---|---|
| 1 | *H&C 899* | Sierra de Juárez, Oax. | 2950 m | 98% | 2% |
| 2 | *H&C 900* | Sierra de Juárez, Oax. | 3000 m | 70% | 30% |
| 3 | *H&C 933* | Nevado de Colima, Col. | 3650 m | 91% | 9% |
| 4 | *VEGA 272* | Río Frio, Mex. | 3050 m | UK | UK |
| 5 | *H&C 914* | Amecameca-Tlamacas, Mex. | 3500 m | 75% | 25% |
| 7 | *H&C 917* | E del Popocatepetl, Pue. | 3180 m | 60% | 40% |
| 13 | *H&C 927* | Lagunas de Zempoala, Mex. | 2900 m | 98% | 2% |
| 16 | *H&C 904* | Nevado de Toluca, Mex. | 3400 m | 60% | 40% |
| 17 | *H&C 935* | Nevado de Colima, Col. | 3740 m | 82% | 18% |
| 18 | *H&C 936* | Nevado de Colima, Col. | 4000 m | 70% | 30% |
| 19 | *H&C 929* | La Marqueza, Mex. | 3100 m | 0% | 100% |
| 20 | *H&C 908* | Sultepec, Mex. | 2100 m | 98% | 2% |
| 24 | *MANCERA 1* | Tulancingo, Hgo. | 2240 m | UK | UK |
| 25 | *H 241* | Sierra de Alcaparrosa, Mex. | 2600 m | UK | UK |
| 35 | *H&C 924* | Ajusco, D.F. | 3350 m | 55% | 45% |
| 37 | *H&C 928* | Lagunas de Zempoala, Pue. | 3170 m | 86% | 14% |
| 39 | *H&C 922* | Ajusco, D.F. | 3260 m | 0% | 100% |
| 40 | *H,P&A 972* | Batopilas, Chih. | 2245 m | 0% | 100% |
| 41 | *H&C 926* | Lagunas de Zempoala, Mex. | 2960 m | 46% | 54% |
| 42 | *H 970* | Batopilas, Chih. | 2105 m | 0% | 100% |
| 46 | *H,P&A 964* | Creel, Chih. | 2380 m | 0% | 100% |
| 47 | *H&A 984* | Sierra de Michis, Dgo. | 2500 m | 99% | 1% |

PCA of the population data (Tables 3 & 4) resulted in complete separation between the species into three groups representing *Muhlenbergia montana, M. quadridentata,* and their putative hybrids with intermediate scores.

Results from pollen analyses (Table 5) have shown individuals with abortive spores for the intermediate forms that overlap with both species. Populations from the mountains of the Trans-Mexican Volcanic Belt (D.F. and México states) and Sierra Madre Occidental (Chihuahua state) contains 100% abortive spores, this supports the position that *Muhlenbergia montana* and *M. quadridentata* are distinct species that interbreed to form sterile intermediates.

The nature of the character differences between the two species also suggests that *Muhlenbergia quadridentata* is not simply an ecological variant of *M. montana*. If it were, we would expect them to differ in features that are strongly susceptible to environmental modification, such as leaf length or overall size. While they do differ in some of these characteristics, the best characters to distinguish *M. quadridentata* from *M. montana* are: The glumes are subequal and truncate, and the second glume is 3-4 toothed to erose in the former; while the glumes are unequal and apiculate, and the second glume is sharply 3-toothed, mucronate to shortly aristate in the latter. Anatomically *M. montana* presents two secondary Vascular bundles (Vb) placed among the primary ones, Vb's are circular in outline, and the girder is present adaxially and abaxially, as mentioned in Herrera-Arrieta & Grant (1994); while *M. quadridentata* presents only one secondary Vb between the primary, the Vb's are elliptical in outline and the girder is present just abaxially. Flavonoid profiles are also good characters to easily separate these two species (Herrera-A. & Bain 1991).

Unfortunately few chromosome counts of these two species were successful in this work, meiotic counts were possible in three of all the collected populations (Herrera-Arrieta 1995), where *Muhlenbergia quadridentata* showed $n$=10, *M. montana* $n$=10 and $n$=20. Attempts to grow these species under greenhouse conditions for mitotic counts were unsuccessful. Earlier published chromosome counts for *M. montana* are $n$=20 (Reeder 1968).

Pollen size varies from 20 to 25 μ in *Muhlenbergia quadridentata* and from 15 to 35 μ in *M. montana*, however, no correlation between the ploidy level and pollen sizes was established among the populations of this work. The differences in ploidy level between these two species validate a generalization from Stebbins (1950) about the relative distribution of diploids and polyploids. This author states that changes caused by polyploidy can often promote the adaptation of the new types to entirely different habitats from those occupied by their diploid ancestors. The polyploidal level shown by *M. montana* combined with its probable hybridization to other species (*M. filiculmis* in the USA and *M. quadridentata* in México) gives a wider pattern of distribution to it.

Our studies have shown a polarized distribution (north, central-south) in the three data sets examined. The geographical distribution of *Muhlenbergia montana* occurs mostly in northern populations, while *M. quadridentata* and hybrid swarms are found in central and southern populations.

## CONCLUSIONS

Recognition of *Muhlenbergia quadridentata* as a species distinct from *M. montana* is supported by this study. The two species differ mostly in glume shape and size, vascular bundle outline and number, flavonoid profiles, and ploidy level. The two taxa differ in some habitat preferences, they never grow in mixed populations, *M. montana* occurs at altitudes ranging between 2100 and 2700 m, in oak and pine forests, and even in mesophytic forest, forming small clumps; while *M. quadridentata* occurs at higher altitudes (up to 4100 m) in pine forests and alpine grasslands, forming big bunches which cover a large area. The hybrids exhibit morphological and anatomical intermediates, and mixed flavonoid profiles. Principal Component Analysis of natural populations of these two taxa demonstrates clear separation between the well defined species with the sterile hybrids intermediate between them. The two groups obtained from the cluster analysis suggest that there has been reduced gene flow between the northern and central-southern populations. The patterns of variation observed in allopatric populations of this species pair at central and southern sites fits the model of production of hybrid swarms summarized in Grant (1956).

## ACKNOWLEDGMENTS

This research was supported by grants from the Instituto Politécnico Nacional and COFAA. Special thanks are given to Isaias Chairez who constructed the PCA diagrams. Appreciation is expressed to the reviewers.

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